REMARKS

The title has been amended so that it is consistent with the title approved by the International Searching Authority. See copy of attached Form PCT/ISA/224, dated December 14, 2000. Claims 34-73 are currently pending. A set of pending claims is attached.

No new matter has been added by way of this Amendment.

Respectfully submitted,

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PATENT
Attorney Docket No. 401544/MATHISEN

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

RATLEDGE et al.

National Phase of PCT/GB00/02695

Art Unit: Unassigned

Examiner: Unassigned

Filed: January 14, 2002

For: CULTURE OF CRYPTHECODINIUM COHNII FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID

AMENDMENTS TO TITLE MADE VIA PRELIMINARY AMENDMENT

Amendment to Title:

~~CULTURE OF CRYPTHECODINIUM COHNII FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID~~ CULTURE OF MICROORGANISMS FOR THE SYNTHESIS OF A POLYUNSATURATED FATTY ACID

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PENDING CLAIMS AFTER ENTRY OF PRELIMINARY AMENDMENT

34. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing a microorganism selected from the group consisting of *Crypthecodinium cohnii* and genetically modified variants thereof with a compound selected from the group consisting of carboxylic acids and carboxylate ions, the microorganism using the compound as a carbon source and synthesizing docosahexaenoic acid.

35. The method according to claim 34, wherein said compound is acetic acid or acetate.

36. The method according to claim 34, wherein the compound is the main carbon source for the microorganism during the culture of the microorganism.

37. The method according to claim 34, wherein the microorganism is cultured in a medium, said use of the compound as a carbon source by the microorganism causing an increase in pH of the medium, and wherein the method further includes, where the compound is a carboxylic acid, addition to the medium of said carboxylic acid, or where the compound is a carboxylate ion, addition to the medium of a carboxylic acid that ionizes to form said carboxylate ion, in response to the increase in pH.so as to decrease the pH of the medium.

38. The method according to claim 37, wherein said addition maintains the pH substantially at a predetermined value.

39. The method according to claim 38, wherein the predetermined value is pH 6.5.

40. The method according to claim 37, wherein the pH of the medium is monitored by means that produces a signal that is used to control said addition to the medium.

41. The method according to claim 40, wherein the signal is used to control addition of one or more of a nitrogen source, a phosphorus source, an amino acid, a vitamin, a salt or another growth factor during the culture of the microorganism.

42. The method according to claim 37, wherein said carboxylic acid or said carboxylic acid that ionizes is added to the medium in a mixture comprising a further compound.

43. The method according to claim 42, wherein the further compound is an organic acid.

44. The method according to claim 42, wherein the further compound is a lipid.

45. The method according to claim 42, wherein the mixture is a waste product from an industrial process.

46. The method according to claim 42, wherein the further compound is a nitrogen source, a phosphorus source, an amino acid, a vitamin, a growth factor, a salt or a lipid.

47. The method according to claim 34, wherein prior to said culture with said compound, the microorganism is grown with said compound.

48. The method according to claim 34, wherein the microorganism is cultured with an organic nitrogen source.

49. The method according to claim 48, wherein the organic nitrogen source is yeast extract and the initial concentration of the yeast extract is greater than 7.5 g/l.

50. The method according to claim 49, wherein the initial concentration of yeast extract is 10 g/l.

51. The method according to claim 34, wherein the microorganism is cultured with a salt or an osmoticant.

52. The method according to claim 34, wherein said culture is performed as a batch process or a fed-batch process.

53. The method according to claim 52, wherein the culture is performed for about 4 to about 10 days.

54. The method according to claim 34, wherein said culture is performed as a continuous process or semi-continuous process.

55. The method according to claim 34, wherein the method further comprises extracting oil including docosahexaenoic acid from the microorganism.

56. The method according to claim 34, wherein the method further comprises purification or partial purification of docosahexaenoic acid from the microorganism.

57. The method according to claim 34, wherein the culture does not include a stationary phase.

58. An oil comprising docosahexaenoic acid prepared from a microorganism cultured in accordance with claim 34.

59. An at least partially purified preparation of docosahexaenoic acid prepared from a microorganism cultured in accordance with claim 34.

60. The method according to claim 34, wherein the initial concentration of the compound is about 8 g/l.

61. A method of culturing a microorganism for the synthesis of docosahexaenoic acid by the microorganism, comprising culturing the microorganism with an organic species comprising an acidic group or an ionized form of an acidic group, the microorganism synthesizing docosahexaenoic acid containing carbon from the species.

62. A method of culturing a microorganism for the synthesis of a polyunsaturated fatty acid by the microorganism, comprising culturing *C. cohnii* with an organic species comprising an acidic group or an ionized form of an acidic group, the C. *cohnii* using the species as a carbon source and synthesizing a polyunsaturated fatty acid.

63. An oil comprising the polyunsaturated fatty acid of claim 62.

64. An at least partially purified preparation of the polyunsaturated fatty acid of claim 62.

65. A microorganism cultured in accordance with claim 34.

66. A microorganism cultured in accordance with claim 60.

67. A microorganism cultured in accordance with claim 61.

68. A method comprising using a microorganism according to claim 65 as a food or a food supplement.

69. A method comprising using a microorganism according to claim 66 as a food or a food supplement.

70. A method comprising using a microorganism according to claim 67 as a food or a food supplement.

71. The method according to claim 51, wherein the salt or osmoticant is sea salt.

72. The method according to claim 53, wherein the culture is performed for about 6 to about 9 days.

73. The method according to claim 55, which comprises purifying the oil to increase the docosahexaenoic acid content of the oil.